STUDIES ON A MECHANISM OF AGE RESISTANCE OF CHICKENS TO THE NEMATODE *ASCARIDIA GALLI*

by

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INTRODUCTION AND REVIEW OF LITERATURE

During the past thirty years much literature has appeared on the immunity elicited by animals to their metazoan parasites. At present, investigators in this field of research agree that helminths are capable of initiating immune reactions much the same as the recognized infectious agents. To date, almost every type of immune reaction has been demonstrated, but since the literature is so extensive, only that concerned with immunity due to age, and the effects of diet on resistance will be reviewed here. A more comprehensive review of the work on the immunity to helminths may be found in the monograph by Taliaferro (1929) which includes all work up to that date, and the compilation by Culbertson of the literature from 1929 to 1938.

Of the different types of immunity elicited by animals to helminths one of the most interesting is age resistance. This type of immunity does not arise as a result of previous or contemporary infection but only as the host grows older. The younger animals of the same species either lack the defense mechanism possessed by older members, or else provide more suitable conditions for the development of the parasite. Those hosts which become infected during the susceptible phase of their life are capable of expelling their parasites when the resistant age is reached.

The phenomenon of age resistance had been observed a number of times before 1911, when the first experimental evidence of this

reaction was obtained. At that time, Looss (1911) observed that in experimental Ancylostoma infections, some of the worms were able to reach maturity in young animals, whereas previous workers had found this almost impossible in old animals. Looss' findings were confirmed by Ransom and Foster (1920) who worked with the ascaris of swine.

Ransom (1921) observed a definite age resistance in chickens to the gapeworm, Syngamus trachea. At about this same time Ackert and Herrick (1928) found that chickens three months of age were much more resistant to the nematode Ascaridia galli than were young chicks. A continuation of this work by Herrick (1926) indicated that the resistance of the chick increased with age up to 105 days, after which there was no further increase.

Herrick (1928) and Scott (1928) working with Ancylostoma caninum infections in dogs and cats found manifestations of age resistance. In older dogs a longer time was found to be necessary for the worms to reach maturity, and the proportion of larvae which developed decreased with age. Sarles (1929a) found that both cats and dogs exhibited an age resistance to A. braziliense. The observations and results obtained by Sarles substantiated Herrick's findings in regard to the relative rate of development of the parasite in susceptible and resistant hosts. Sarles (1929b) found that dogs which had harbored hookworms for some time were more resistant to infection than were young animals. He reasoned that most of this resistance was due to age, and that a minor part of it may be acquired.

Ackert, Porter, and Beach (1935) in a quantitative study of the age resistance of fowls to A. galli found that the resistance developed very rapidly; that a difference in age of one week definitely inhibited the growth of the worm.

Clapham (1934) found little evidence of age resistance in chickens to the cecal worm Heterakis gallinae. However, Winfield (1933) working with a closely related species of worm, Heterakis spumosa in rats, found conclusive evidence of age resistance. This resistance apparently influenced the infectivity and development of the worm, and the egg production of the parasite.

That laboratory rats may exhibit age resistance to Nippostrongylus muris was observed by Africa (1931) and Chandler (1932). Sheldon (1937) observed that the degree of age resistance manifested by rats toward Strongyloides ratti was not as high as that of the corresponding hosts toward Ancylostoma caninum, A. braziliensi, and Ascaridia galli, but did approach that of rats to N. muris.

The fact that many animals may exhibit age resistance toward their helminthic parasites does not necessarily mean that all hosts do, likewise, the term "age resistance" may not in certain cases be applicable. Sarles and Stoll (1935) postulated that the term as applied to the cat in regard to Toxocara cati seemed unwarranted since kittens were found to be as refractory as old cats. Moreover, experimental evidence for both age resistance and active acquired resistance was negative. Foster (1935) in an experimental study of the immunity of dogs to hookworm obtained data which indicated that the resistance of the dog to A. caninum is

neither a true acquired immunity, nor fundamentally an age resistance. The emphasis instead was placed on the animals' general condition of health as the regulating factor in the development of resistance.

A survey of 1315 dogs in the New Orleans area by Hinman and Baker (1936) yielded little evidence of age resistance to certain parasites. These workers observed that the frequency of Ancylostoma infections in immature and mature dogs was so similar as to suggest that age resistance to natural infection was of no particular significance. There was, however, a definite resistance to Toxocara infections.

Otto and Kerr (1939) were unable to find any conclusive evidence that age resistance existed as an entity in itself. These workers based their conclusions on the assumption that maturity is only one of the factors contributing to the well being of the host animals which enable it to respond to the invading organism.

The study of the factors involved in the resistance of animals to their helminth parasites has been quite extensive. Most of these studies, however, have been of a dietary nature. Ackert (1927) in an examination of 1000 chickens on farms near Manhattan, Kansas, found 49 percent of them infected with A. galli. However, an examination of 500 chickens reared in confinement, all of which had been parasitized, showed only a 28 percent infection. Reasoning that a scarcity of green feed was a factor in the lowered resistance of the farm chickens, subsequent tests run under experimental conditions showed that a deficiency of Vitamin

A significantly lowered the resistance of chickens to the nematode, *A. galli* (Ackert, McIlvaine, and Crawford, 1931). A deficiency of Vitamin B complex was also found to lower the resistance of the chickens (Ackert and Nolf, 1931). Tests run by Ackert and Spindler (1929) showed that while Vitamin D does not appear to be a factor in the resistance of chickens to *A. galli* it does serve to protect the host against severe effects of the parasite.

Foster and Cort (1932a) in a study of the relation of diet to *A. caninum* infections in dogs found a definite correlation between undernourishment and susceptibility to the parasite. A definite break in resistance was found in the undernourished animals which previously had developed a high degree of resistance by virtue of age and previous infection. A possibility that a relationship exists between undernourishment and incidental parasitism was found by Foster and Cort (1932b).

Work by Ackert and his associates has indicated that a full volume of blood (Ackert, 1926) and certain protein supplements (Ackert and Beach, 1933) are also factors in the development of resistance.

In addition to these studies, work by Chandler (1932), Ross (1932), Wright (1933), Spindler (1933) and McCoy (1934) in all cases indicated a relationship between the resistance of an animal to helminthic infection and a deficient or inadequate diet.

Age resistance is usually not complete. It is more generally manifested in a retardation of development of the larval parasite, a lowering of the degree of infection, and a reduction in the egg production of the adult parasite. Several hypotheses have been

proposed for the possible mechanism of age resistance, all based on direct, and probably specific effects on the parasite. Sarles (1929c) in daily blood studies on young and old dogs during infection with _Ancylostoma_ found that in old dogs there was an immediate decrease in the number of eosinophiles following infection, succeeded by a gradual increase which produced an eosinophilia of 17 to 40 percent on the 10th to 14th day after infection. There was also a definite increase in the total number of leucocytes in the blood. This did not occur in young, susceptible dogs. In this case the mechanism of age resistance would be based on blood changes in the host.

Ackert, Porter, and Beach (1935) proposed that since the explanation of the results obtained in age resistance studies on the chick to _A. galli_ did not lend itself to the abnormal host theory postulated by Sandground (1929), another hypothesis must be adopted. At that time the proposal was made that as the chick ages its body develops certain growth inhibiting factors which react against the development of the nematode. A mechanism of this type is also suggested by Chandler (1936) as being responsible for the resistance of rats to _Nippostrongylus_.

Until this time all resistance studies had been carried out under _in vivo_ conditions. Such studies would be greatly facilitated if they could be carried out _in vitro_. In 1938, Ackert, Todd, and Tanner reported on a technique by which it was possible to culture the nematode _A. galli_ artificially in an isotonic-salt-dextrose medium. These workers were able to induce growth ranging up to 53 percent in young _Ascaridia_ _in vitro_. Previous

to this work, studies by Ackert and Whitlock (1935) and Ackert and Freeman (1936) indicated that the growing Ascaridia derived its nourishment from the contents of the lumen of the hosts' intestine rather than from the epithelium.

In 1938, Ackert and Edgar reported on a series of histological studies on the intestinal habitat of A. galli. Observing that the number of goblet cells per unit area of intestine increased directly with the age of the chicken, these workers postulated that there might be some relationship between the increase in number of goblet cells and the development of age resistance. Accordingly, tests run in which duodenal mucus from resistant chickens was introduced into cultures of the worms indicated that this mucus contained some factor which inhibited the growth of the nematode, A. galli (Ackert, Edgar, and Frick, 1939).

Following this study more exhaustive tests were run in an effort to determine the role played by goblet cells and duodenal mucus in the phenomenon of age resistance of chickens to Ascaridia galli.

MATERIALS AND METHODS

Technique of Parasitizing

All the fowls used in this problem were obtained as day-old chicks from a commercial hatchery at Wichita, Kansas. Immediately upon arrival the chicks were transferred to electrically heated brooders and put upon a diet which had been tested and approved by the Kansas State College Poultry Department. Seven to ten days

after arrival, the chicks were transferred to fly-proof pens. From this time on the fowls were kept on clean straw and always had an adequate supply of feed and water.

The chickens used in these experiments were parasitized at 14 to 21 days of age by feeding with embryonated nematode eggs. These eggs were cultured artificially in sterile distilled water after the method described by Ackert, Eisenbrandt, et al (1935).

Due to the fact that the effects of the worms on the chickens were not being studied, and that the fowls were being used only as a source of worms and mucus, the number of eggs fed the chicks were not counted accurately, although an effort was made to keep the number approximately constant.

Recovery of the Mucus

All samples of the mucus used in this problem were collected in essentially the same way. Immediately after the chicken was killed, the body was opened and the portion of the small intestine (duodenum) between the point of entrance of the bile ducts and the yolk sac diverticulum was removed, since this portion of the small intestine is the usual habitat of the worm. Therefore, only the mucus from this region was used. The mucus was obtained by slitting the intestine on the side opposite the mesenteric connection and then gently scraping the mucosa with a clean, blunt spatula. The process of scraping was done as rapidly as possible to prevent overlong exposure to air and to lessen the opportunity for autolysis. The whole procedure of mucus recovery was completed in from 6 to 9 minutes. Any debris or undigested food material in the lumen was removed.

Since the mucus was used differently in each of the several tests within each series of experiments, the specific treatment of it will be described as each series is considered. Reference will be made to "young mucus" and "old mucus". These terms are used to indicate mucus from young and old chickens, respectively.

Recovery of the Worms

All worms were recovered in the same way. After the intestine was slit, the worms were floated off into sterile saline which had been kept at a temperature corresponding to the body temperature of the host, that is, 106 - 108° F. The worms were allowed to wash approximately an hour before being used. They were transferred with a hooked needle, and care was taken to handle them as little and as carefully as possible. These precautions were necessary in order that the protective mucous covering of the worm be not broken, for if this occurred, the worms became extremely susceptible to mold and fungous attacks.

Culture Media

The original medium used by Ackert, Todd, and Tanner (1938) in the artical culture of Ascaridia galli had the following formula:

NaCl	2.25 gm.
$CaCl_2$	0.06 gm.
KCl	0.10 gm.
$NaHCO_3$	0.04 gm.
Dextrose	0.62 gm.
H_2O	1000 cc.

This solution was used over a base of cornmeal agar, but in the present work it was found that this base made the culture

extremely favorable for molds and fungi, and that it could be omitted. The above medium was used in all of the preliminary tests, and in the comparative study on autoclaved mucus.

Although this medium as used by Ackert, Todd, and Tanner gave very good results, it had certain disadvantages, one of which was that the formula had to be diluted with four volumes of distilled water before use. Since the original total ionic concentration of the medium had not been calculated, the arbitrary addition of water without subsequent check tests would give only a rough estimate of the isotonicity of the medium for A. galli.

In 1939, Fenwick reported on partial results obtained in his studies on the artificial culture of Ascaris larvae. The medium used by Fenwick had the following formula:

$NaCl$	0.80%
$CaCl_2$	0.02%
KCl	0.02%
$MgCl_2$	0.01%

This formula had a total ionic concentration equivalent to a 0.832 percent saline solution. This Fenwick had determined by a series of tests, to be isotonic for Ascaris larvae. It had also been found that the larvae could maintain themselves in this medium with dextrose added in concentrations up to 0.1 percent provided that the concentration of NaCl was adjusted to keep the total ionic concentration at 0.832 percent.

Since Ascaris suum and Ascaridia galli are quite similar, tests were run in which Fenwick's medium was substituted for the original medium. The solution was tested with concentrations of dextrose of 0.0155 percent, 0.050 percent and 0.100 percent. In each case the concentration of NaCl was adjusted so that the total

ionic concentration remained at 0.832 percent. As nearly as could be determined, the above concentration was also isotonic for A. galli. While each of these three concentrations of dextrose gave good results, on the average, the addition of dextrose to 0.05 percent gave the best results.

The original pH of the above formula was 7.0 which was well within the optimum range for the parasite. The addition of mucus to the solution often necessitated an adjustment of the pH. All pH determinations were run on hydroquinone and glass electrode potentiometers.

Ackert, Todd, and Tanner used as a check on their nutrient solution a 0.9 percent saline solution. This solution was also used in all preliminary tests, but in later tests, Fenwick's base solution without the addition of dextrose was used.

Measurement of the Parasites

The original procedure for the measurement of the worms consisted in magnifying the nematodes six diameters by the use of a photographic bellows, and then tracing the outline of the worm on onion skin paper. The length of the worms could then be found by measuring the outline with a calibrated wheel. Previous to this work, this procedure was used in all work in which linear measurements were involved.

This method had several serious disadvantages. The worm was often exposed to room temperatures for several minutes; it was subjected to drying; the worm was often handled too much; and moreover, it was extremely difficult to trace the outline of the

active worm. Consequently, for the present work, a modification of the former method was developed. The worms were first photographed against a non-reflecting background on 35 mm. film. A six-diameter enlargement of the photograph was made on onion skin paper and the outline of the worm traced. Then, by multiplying this length by the reducing power of the camera, which, with the lens system used, was calculated to be 3.33, the actual length of the worm could be found. Advantages of this method were that the worm was not subject to drying, cold, or unnecessary handling, and that the measurements were extremely rapid and accurate. After death the nematodes were measured directly by the use of the photographic bellows.

EXPERIMENTAL DATA

The results obtained by Ackert and Edgar (1938) on the histological study of the intestinal habitat of the parasite indicated that the number of goblet cells per unit area increased directly with the age of the chick. It was also noted that this increase in number of goblet cells coincided very closely with the natural development of age resistance of the host to the parasite.

The principal function of the goblet cells is the secretion of mucus, which is regarded by most authorities as a lubricant for the intestine. Mucin, probably the most important constituent of mucus, is a glyco-protein generally considered to be a factor in the prevention of auto-digestion of the mucosa.

The larger numbers of goblet cells in older chickens naturally led to the inference that a relation might exist between the increased volume of mucus and the resistance of the chickens to the nematodes. It would perhaps be logical to assume that since the older chickens had a larger volume of mucus, the efficiency of the mucus as a lubricant increased by the excess amount of that material would make it difficult for the worms to maintain themselves, and many would be eliminated. Since the normally unattached worms could only with difficulty maintain themselves in such a host, this host would be said to be resistant. By this hypothesis, the relation between the increase in number of goblet cells per unit area in older chickens and the development of resistance to A. galli is placed on a quantitative and mechanical basis. Undoubtedly this postulation has certain values, but it would not explain the results found by Herrick (1926) which showed that for the first ten days of infection the worms in chicks five days of age attained a mean length of 5.6 mm., whereas worms for the same period in chickens 140 days of age gained only 0.4 mm. These results indicate that the relationship may be based on qualitative and chemical bases.

Comparative Study with Autoclaved Mucus

To determine in vitro effects of mucus on the intestinal worm A. galli, some preliminary tests were run. The technique was as follows: A 90-day chicken was killed and the mucus collected and autoclaved as outlined under materials and methods. The autoclaved material was then added to the worm culture. The ratio

in each case between mucus and nutrient medium was 3:65. From three trials, results were obtained as shown in Table 1.

Table 1. Results of tests for an inhibitory nematode growth factor in duodenal mucus from chickens three months old. (From Ackert, Edgar, and Frick, 1939)

Culture media	Larvae in culture days	Gain in length average (mm.)	Percent gain
	Experiment I		
*Isotonic-salt-dextrose solution plus mucus	3.0	2.2	4.0
Isotonic-salt-dextrose solution (control)	2.5	11.8	18.5
Isotonic-salt solution (no nutriment)	2.0	2.5(loss)	0.0
	Experiment II		
Isotonic-salt-dextrose solution plus mucus	3.0	8.5	12.8
Isotonic-salt-dextrose solution (control)	3.0	27.0	36.9
Isotonic-salt solution (no nutriment)	3.0	.2(loss)	0.0
	Experiment III		
Isotonic-salt-dextrose solution plus mucus	3.0	2.1	2.9
Isotonic-salt-dextrose solution (control)	3.0	14.3	19.8
Isotonic-salt solution (no nutriment)	4.0	11.0(loss)	0.0

*The pH of the isotonic-salt-dextrose solution was 6.1; that of the autoclaved mucus was 6.0.

These data indicated that while the worms in the nutrient solution were able to increase their length from 18.5 to 36.9 percent, the worms in the cultures to which mucus had been added, even though in the presence of nutriment, grew only 2.9 to 12.8 percent. From the results of these trials it appeared that duodenal mucus

from resistant chickens contained some factor which was unfavorable for the growth of A. galli.

Even though the addition of 90-day mucus to the culture had inhibited the growth of the worms, there was no definite proof that this inhibition might not have been due to some mechanical reaction resembling crenation, brought about by a change in the concentration of the medium with the addition of the mucus. In order to check this possibility, another test was run in exactly the same manner as the previous tests except that in this case mucus from a 45-day chick was used. The results of this test in which the worms grew as well in the presence of the mucus as in its absence, indicated that mucus from 45-day chickens lacked the power to retard the growth of the worms (Table 2). Since mucus from 90-day chickens appeared to have an inhibitory power against the worms and mucus from 45-day chickens lacked this ability, it seemed desirable to test mucus from chickens of various ages, especially that from chickens ranging from 45-day to three or four months of age. From several such tests, the results which are shown in Table 2, indicated that there was a progressive increase in the potency of the mucus as an inhibitor as the age of the chicken increased. For example, the worms in Group B in the presence of mucus from 54-day old chickens gained an average of 17.916 mm. as compared with 21.74 for the control worms without mucus; and in Group H the worms in the presence of mucus from 125-day chickens failed to gain and even lost 4.49 mm. in length while the controls in the absence of the mucus gained 9.02 mm. From the results of these tests it is seen that be-

Table 2. *A. galli* cultured in varying ages of autoclaved mucus.

Worms Group	Age of Mucus (days)	Experimental Worms Ave. Gain (mm.)	Experimental Worms Ave. Loss (mm.)	Controls Ave. Gain (mm.)
A	45	9.598		9.205
B	54	17.916		21.740
C	63	14.406		17.090
D	66	13.555		15.880
E	70	12.090		42.380
F	107	4.750		28.530
G	117		0.83	8.250
H	125		4.49	9.020

ginning with mucus from chickens 54 days of age, the growth of the worms was retarded progressively to the age of at least 125 days.

Comparative Study with Unautoclaved Mucus

The results of the tests described thus far have been indicative, but not conclusive, primarily because the numbers of samples used were not large enough to obtain a good estimate. Consequently a much larger experiment was so planned and arranged that it would be possible to test biometrically worms between the ages of 30 and 90 days, taken by 10-day intervals, with mucus from chickens 45 to 135-days of age also taken by 10-day intervals.

The procedure of this experiment was somewhat different from those of the preceding tests. In the previous tests the mucus was autoclaved before being used. The fact that inhibition occurred indicated that the inhibitor is not of the nature of an antibody. However, it was necessary to run tests with both autoclaved and unautoclaved material, so that one series of tests might serve as a check on the other. In all tests in the experiment under consideration the mucus was used in the untreated condition.

The cultures were made very much as they were in the experiments on autoclaved mucus except for one major difference. Certain preliminary trials run before the principal tests showed that it was possible to culture a number of worms in one container provided that each had a sufficient quantity of medium.

The main disadvantage of this technique lay in the fact that individual records of the worms could not be kept. But since the worms varied to some extent in their reaction, and since the outlay of work and expense in order to keep individual records of any large number of worms would have been prohibitive, it was decided to run, as far as possible, ten worms in each lot, and to consider only the mean gain or loss of this group of worms in the analysis. Ten worms were found to give an accurate estimate of the variability of the worms. However, in some of the tests in the second half of the experiment it was necessary to use smaller worm samples. This necessitated recalculating all of the data on a per-worm basis.

Since all the factors in the experiment were controlled except the mucus samples, it was necessary to run only one series of controls. This series was run early in the experiment on worms between 30 and 90 days of age, taken by 10-day intervals. The data on the control tests are given in Table 3. As far as was possible, each group in the series was matched by a group of worms cultured in the isotonic, non-nutrient medium.

The medium used in all tests is the one based upon Fenwick's solution which was described earlier.

The chickens which were used as a source of worms and mucus were divided into six main groups, each of which was divided into two sub-groups. In general, the worms from one sub-group were cultured in mucus from the other; the worms from that sub-group in turn being cultured in mucus from the first. The difference in ages between the two sub-groups in each main group were made to follow a definite interval.

Table 3. Control and check series *A. galli* cultured *in vitro*. Control worms cultured in nutrient medium; checks in non-nutrient medium.

Group	Age	Controls			Checks		
		Mean Init. Length (mm.)	Mean Final Length (mm.)	Mean Gain (mm.)	Mean Init. Length (mm.)	Mean Final Length (mm.)	Mean Loss (mm.)
A_1	30	38.293	49.566	11.273	40.857	39.875	0.982
A_2	30	47.298	62.816	15.518			
B_1	40	41.402	45.831	4.429	41.301	37.830	3.471
B_2	40	55.716	63.996	8.280			
C	50	66.660	74.100	7.440	61.212	59.633	1.579
D	60	81.747	102.716	20.969	61.102	53.448	7.654
E	70	81.471	89.400	7.929			
F_1	80	90.561	100.216	9.655			
F_2**	80	62.576	67.733	5.157			
F_3**	80	60.964	68.882	7.918			
G_1	90	92.350	107.665	15.315			
G_2**	90	66.762	71.649	4.887			
G_3**	90	71.088	75.582	4.494			

**Males only.

If any effects were to be exerted by the mucus, they would show in at least three days. Therefore the cultures were allowed to run for this length of time, after which the worms were killed in hot A.F.A. solution.

A general summary of the experimental data is given in Table 4. A test of significance by analysis of variance run on the data as given in this table proved to be nonsignificant indicating that either there was no difference between the different samples of mucus when considered by 10-day intervals, or that the interaction of the worms was so high that it obscured any actual difference.

Therefore, the data were regrouped as in Table 5, by putting the experiment on a 20-day basis and considering ages 55, 75, 95, 115, and 135 as replicates of ages 45, 65, 85, 105, and 125 respectively. The 40-60-day group was deleted since the results of this group were entirely out of line with the rest of the groups. This aberration was thought to be due to some factor other than the one being studied, entering the experiment, for when the group totals were considered, that of the 40-60-day group was greater than any of the others except the 120-140-day group. This might possibly indicate that some other mechanism was established soon after parasitizing and that it existed only a relatively short time.

A summary of the regrouped data is given in Table 5 and the outline of the analysis of variance for these data in Table 6. With the deletion of the first group, the difference between the age groups of mucus proved to be highly significant. The F

Table 4. Summary of a test to determine the comparative inhibitory ability of duodenal mucus of varying ages on varying ages of <u>A. galli</u>. Mucus not autoclaved. Data on per-worm basis.

Age of Worms	Age of Mucus in Days									
	45	55	65	75	85	95	105	115	125	135
30	+1.2270	-0.3140	-0.4680	-0.8340	+0.9856	-0.9350	-1.4610	-1.1650	-1.5740	-0.3610
40	-0.1120	+0.8860	+0.1030	-0.4602	-0.1730	-1.6030	-0.9156	-0.1397	-0.7140	-0.5310
50	-0.2686	+0.1780	-0.0008	+0.3636	-1.4261	-0.9981	-0.6766	-0.5820	-2.9303	-0.1870
60	-1.5488	-0.8001	+0.6100	+0.8080	-0.3454	-2.8212	-0.3912	-2.8076	-1.5270	-1.6954
70	-0.5955	-0.4253	-0.9360	+0.3595	+0.4430	-0.3726	-1.4921	-1.5300	-0.8577	-2.5899
80	-3.3060	+0.7170	-0.7511	-2.3630	-0.1641	-0.1813	-0.3920	+0.7290	-2.2370	-3.9514
90	-3.1090	-6.0250	-0.7525	-1.9920	-1.8480	-0.6476	-0.0892	-0.8578	-2.9670	-6.9690

Table 5. Summary of data on per-worm basis of *A. galli* cultured in untreated mucous of varying ages.

Age of Worms	Trial	Age Groups of Mucous A 60-80	B 80-100	C 100-120	D 120-140	Sums
30	1	-0.468	+0.9856	-1.461	-1.574	-2.5174
	2	-0.834	-0.9350	-1.165	-0.361	-3.2950
	Sum	-1.302	+0.0506	-2.626	-1.935	-5.8124
40	1	+0.1030	-0.173	-0.9156	-0.714	-1.6996
	2	-0.4602	-1.603	-0.1397	-0.531	-2.7339
	Sum	-0.3572	-1.776	-1.0553	-1.245	-4.4335
50	1	-0.0008	-1.4261	-0.6766	-2.9303	-5.0338
	2	+0.3636	-0.9981	-0.5820	-0.1870	-1.4035
	Sum	+0.3628	-2.4242	-1.2586	-3.1173	-6.4373
60	1	+0.610	-0.3454	-0.3912	-1.5270	-1.6536
	2	+0.808	-2.8212	-2.8076	-1.6954	-6.5162
	Sum	+1.418	-3.1666	-3.1988	-3.2224	-8.1698
70	1	-0.9360	+0.4430	-1.4921	-0.8577	-2.8428
	2	+0.3595	-0.3726	-1.5300	-2.4899	-4.1330
	Sum	-0.5765	+0.0704	-3.0221	-3.5476	-6.9758
80	1	-0.7511	-0.1641	-0.392	-2.2370	-3.5442
	2	-2.3630	-0.1813	+0.729	-3.9514	-5.7667
	Sum	-3.1141	-0.3454	+0.337	-6.1884	-9.3109
90	1	-0.7525	-1.8480	-0.0892	-2.9670	-5.6567
	2	-1.9920	-0.6476	-0.8578	-6.9690	-10.4664
	Sum	-2.7445	-2.4956	-0.9470	-0.9360	-16.1231
	Sums	-6.3135	-10.0868	-11.7708	-29.0917	-57.2628
	Group Means	-0.902	-1.441	-1.681	-4.156	

Table 6. Analysis of variance for effect of duodenal mucus of varying ages on A. galli.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	55	97.0468	
Worms	6	11.0626	1.8438
Mucus	3	21.9089	7.3029**
Worm-Mucus interaction	18	34.0804	1.8900
Trials	1	2.3071	2.3071
Worm-trial interaction	6	6.2224	1.03706
Mucus-trial interaction	3	0.6911	0.2304
Worm-Mucus-trial interaction	18	20.7743	1.1500

**Highly significant: F = 7.3029/1.15 = 6.35 surpasses the 1 percent point, 5.09, for 3 and 18 degrees of freedom.

value, which in analysis of variance is the point upon which significance is determined, is found by dividing the greater mean square by the lesser mean square (Table 6). The data given in this experiment gave the highly significant F value of 6.35 which surpassed the 1 percent level of significance, 5.09, for 3 and 18 degrees of freedom.

Thus far a highly significant difference has been shown to exist between the different ages of mucus involved in this experiment. In order to determine where this difference lies a T test was applied to the group means using data derived from the analysis of variance. This test showed that while there was no significant progressive difference between the 60-80, 80-100, and 100-120-day groups, there was a highly significant difference between the 120-140-day group and any other group. Referring to Table 5, it will be seen that the group means are as follows: Group A, -0.902; Group B, -1.441; Group C, -1,681; and Group D, -4.156. For the 5 percent level of significance, a difference of 1.20 was necessary, and for the 1 percent level, 1.64. Thus it is readily seen that the difference between Group D and any other group was highly significant.

MUCUS EXTRACTION

The results of tests made with both autoclaved and unautoclaved whole mucus, introduced into cultures of the worms, have shown conclusively that duodenal mucus from resistant chickens contains some factor which has the ability to inhibit the growth of <u>A. galli</u>.

This duodenal mucus is a heterogeneous mixture of mucin and other chemical constituents, as well as varying amounts of partially digested food material, and undoubtedly certain tissue fragments.

The next step in the study was to attempt to determine in which fraction of the mucus the active principle is contained.

A test was therefore set up in which mucus from birds 171 days of age was recovered and washed with two 30 cc. portions of sterile saline. The mixture was centrifuged between each washing, and the supernatant liquids saved. The washed mucus was ground and extracted with 60 cc. of sterile saline. The washings and the extract were added to the nutrient solution in the usual ratio of 3:65, the suspensions autoclaved, the pH adjusted, and the worms added.

The mixtures were autoclaved for two reasons: first, the results obtained with autoclaved material in the comparative study were almost identical with the conclusive results obtained with the untreated mucus; and second, maintaining the sterility of the unautoclaved mucus entails a great deal of effort. Thus the efficiency of the experiment could be increased by autoclaving the mucus before use.

The results of this test, which are shown in Table 7, indicated that the inhibitory factor exists in either two phases: one of which is soluble in 0.9 percent saline, and the other insoluble; or that it is entirely soluble and only appeared in the extract because it was not entirely leached out of the solid fraction. The worms cultured in the presence of the washings

The fact that an actual qualitative difference between young and old mucus does exist has been demonstrated in this problem; the data being supported by statistical analysis. It has also been shown that the factor which differentiates between old and young mucus is not, when the problem is considered as a whole, of the nature of an antibody, since it is not destroyed by autoclaving. This fact detracts from the idea that the immunity exhibited by old chickens to A. galli is of the acquired type; dependent upon previous or contemporary infection. This type of immunity must necessarily be based upon an antigen-antibody mechanism, and this mechanism is quite readily destroyed by heat.

The results from the study in this respect offer support to Chandler's hypothesis (1939) that helminths which do not enter the body proper, but pass their lives as parasites in the lumen of the digestive tract, seem to have little or no ability to stimulate the host to produce immune substances. These results also substantiate the work of Eisenbrandt and Ackert (1940) who found that serological data were negative for chickens which had been previously parasitized.

It is seen in the summary of data on the comparative tests with autoclaved mucus (Table 2) that the growth rate of the worms in some of the groups, particularly Groups B and E was abnormally high. This may have been due to one of three things: first, the culture medium was not exactly isotonic for the worms; second, the worms contained a rather high amount of reserve food material when they were transferred to the cultures; or third, the worms at the time they were put into culture were at a point

Table 7. A. galli cultured in 171-day autoclaved mucous washings and mucus extract.

No.	Culture	Days in	Length in (mm.)	Length out (mm.)	Gain in Length (mm.)	Loss in Length (mm.)
11D1	Iso-salt plus	6	66.30	77.00	10.70	
11D2	dextrose	6	56.10	60.00	3.90	
11D3		$5\frac{1}{2}$	68.00	75.00	7.00	
11D4		6	71.40	74.00	2.60	
				Ave. gain	6.05 mm.	
11W1	Nutrient plus mucus	9	76.50	99.25	12.75	
11W2	washings	8	73.10	67.66		5.44
11W3		9	64.60	62.50		2.10
11W4		8	73.64	64.00		9.64
				Ave. loss		1.11 mm.
11G1	Nutrient plus ground	8	66.30	59.50		6.80
11G3	mucus extract	8	73.10	59.50		13.60
11G4		$6\frac{1}{2}$	57.80	63.50	6.70	
				Ave. loss		4.57 mm.

exhibited an average loss of 1.11 mm., while those in the extract lost 4.67 mm. This would indicate that the fraction of the inhibitor present in the extract is the more potent. The control worms gained an average of 6.05 mm.

A second test was run on a slightly different basis. Two 115-day-old chickens were killed and approximately 12 cc. of mucus recovered. The mucus was added to 240 cc. of the nutrient medium and the mixture allowed to stand nine days at refrigerator temperature. This procedure it was hoped, would determine whether the factor was entirely contained in the soluble fraction of the mucus, or whether part of it was contained in the insoluble portion. During the nine days the mixture was left standing, any soluble substances should be leached out.

After the nine-day interval, the mixture was centrifuged and the supernatant liquid decanted. On the basis of a previous trial run with mucus from 97-day-old chickens, it was found that of 5 cc. of raw mucus recovered, 4 cc. were soluble and 1 cc. insoluble. In the case now under consideration the ratio between soluble and insoluble fractions was doubled in order to allow for the presence of debris in the mucus. On the basis of both this ratio and the quantity of liquid recovered, the soluble portion of the mucus was calculated to be in a concentration of 1:35.

Due to loss in transfer, only 2 cc. of the calculated 4.8cc. volume of solids were recovered. The insoluble material was added to 75 cc. of the nutrient solution, making a dilution of 1:38.

Five 70-day worms of approximately the same size were measured and put into the 75 cc. of the 1:38 dilution of solids and 75 cc. of the 1:35 dilution of washings, respectively. The cultures were allowed to run three days after which the worms were killed in hot A.F.A. solution, and remeasured.

The results of this test were quite different from those of the preceding tests of the same type. In the test under consideration, the worms in the 1:38 dilution of solids showed an average gain of 1.482 mm. per worm, a gain approximately equal to what would be expected from 70-day worms, whereas the worms in the 1:35 dilution of the soluble fraction showed an average loss of 1.48 mm. per worm. Thus, the gain in one was equal to the loss in the other.

The inference from this test is that the growth-inhibiting factor is contained only in the soluble fraction of the mucus. It is highly improbable that the portion of the insoluble material that was lost could have contained any of the inhibitor, for the mucus-medium mixture was thoroughly shaken twice a day during the period of washing.

Titration of the Washings

The experiment described above gave conclusive evidence that the inhibitory factor is entirely contained in the soluble fraction of the mucus.

In order to determine the effect that dilution might have on the efficiency of the inhibitor, a titration was run with dilutions ranging from 1:35 to 1:2240. Four eighty-day worms

were cultured in each of the dilutions. The results of this test are shown in Table 8 and Fig. 1.

It will be seen from the results of this experiment that the efficiency of the inhibitor is almost directly dependent upon concentration.

Effect of Filtration on Washings and Extract

In the preceding tests, it has been shown that the growth inhibitor contained in duodenal mucus taken from resistant chickens which had been previously parasitized is contained in the fraction of the mucus soluble in 0.9 percent saline.

Following these tests an experiment was run in which the washings and the extract of mucus taken from resistant chickens which had not been parasitized were filtered before use.

This test was designed to show two things: First, the effect that mucus from unparasitized birds might have on the worms; and second, the effect that filtration might have on the washings and the extract.

The technique of mucus recovery, washing, and extraction in this test was the same as in the previous test on extraction. After extraction, the extract and the washings were filtered through a Berkefeld candle.

The data on this test are shown in Table 9.

In general, the results of this test were quite comparable to those of the first test on extraction, and in addition indicated that mucus from unparasitized chicks of the age ordinarily considered resistant also contained the inhibitory factor, and furthermore, that the factor was filterable.

Table 8. Titration of unautoclaved mucous washings.

Dilution	Loss of Length of Worms (mm.)	Gain in Length of Worms (mm.)
1 - 35	7.400	
1 - 70	2.493	
1 - 140	1.138	
1 - 280	2.990	
1 - 560		1.460
1 - 1120		1.595
1 - 2240		4.525

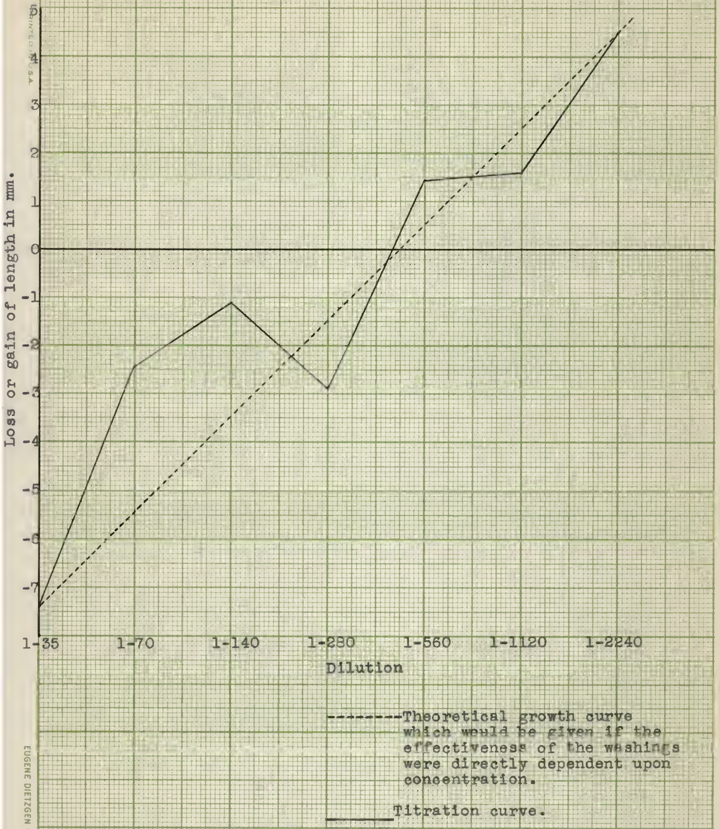

Fig. 1. Titration of mucus washings. Mucus not autoclaved. Age of mucus, 115 days; worms, 80 days.

Table 9. Effect of filtration on washings and extract of mucus from 90-day unparasitized chickens.

No.	Culture	Days in	Length in (mm.)	Length out (mm.)	Gain in Length (mm.)	Loss in Length (mm.)
13D1	Regular nutrient	4	65.32	73.00	7.68	
13D2	medium	3	62.00	79.50	17.50	
13D3		5	64.00	57.66		6.34
				Ave. gain	6.28 mm.	
13G1	Nutrient plus ppt.	3	76.00	70.00		6.00
13G2	from filtered	3	68.00	62.00		6.00
13G3	ground mucus	3	78.64	74.00		4.64
				Ave. loss		5.55 mm.
13F1	Nutrient plus filtrate	5	78.00	84.66	6.66	
13F2	from filtered mucus extract	3	66.00	76.00	10.00	
13F3		4	80.00	104.50	24.50	
13F4		4	90.00	90.00	0.00	
				Ave. gain	10.29 mm.	
13W1	Nutrient plus	5	48.00	40.66		7.34
13W2	filtered mucus	4	58.00	50.50		7.50
13W3	washings	3	60.00	60.00		0.00
13W4		3	46.00	52.00	6.00	
				Ave. loss		2.21 mm.

This test, as did the first test of this series, indicated that part of the factor is contained in the insoluble fraction of the mucus. However, the second test of this series gave conclusive evidence that the inhibitory ability is contained only in the soluble fraction. In neither of the two cases in which two phases of the inhibitor were evident was the mucus allowed to set long enough to complete the leaching process.

Recovery Tests

In all the tests considered thus far, the effect of mucus from resistant fowls on the worms had been manifested in the inhibition of growth of the worms. This fact suggested that the inhibitory factor in the mucus interfered in some way with the normal nutritional processes of the worm. However, it was not known whether the effects exerted by the factor were of a permanent or temporary nature.

In order to test this hypothesis, a test was run in which worms, after being subjected to the actions of mucus for a certain period of time, were remeasured and transferred to the regular nutrient solution. The results of this test are shown in Table 10, Trial 1.

From these data it will be seen that the nematodes, after being cultured eight to nine days in the presence of the factor, had, with one exception, the ability to recover and resume growth when transferred from the mucus cultures to the nutrient solution. In each of the cases where recovery occurred, the worms were able to make up any loss of length resulting from the mucus treatment, and in addition, to add extra growth. The loss of length after

Table 10. Recovery of worms after transfer from mucus cultures to nutrient medium.

No.	Days in mucus culture	Length out of mucus culture (mm.)	Length out of nutrient culture (mm.)	Days in nutrient	Total days	Gain in nutrient (mm.)	Loss in nutrient (mm.)	Gain or loss in mucus culture (mm.)
				Trial 1				
11G1	8	59.50	77.00	5½	13½	17.50		- 6.80
11G3	8	59.50	81.00	6	14	21.50		-13.60
11W1	9	89.25	85.00	4	13		4.25	+12.75
				Trial 2				
13G1	3	70.00	88.50	6	9	18.50		- 6.00
13G2	3	62.00	62.50	2	5	0.50		- 6.00
13G3	3	74.00	79.50	1	4	5.50		- 4.64

in the 11W1 culture may have been due to some mechanical injury to the worm, or it may have been an expression of the inherent variability of the worms.

The nematodes used in this test were part of those used in the experiment on extraction.

This experiment was repeated on some of the worms used in the test on the effects of filtration. These results are shown in Table 10, Trial 2. These data were very much like those of the previous test. In the experimental cultures the worms suffered an average loss of 5.55 mm. After transfer the worms gained an average of 8.17 mm. which made up the loss incurred in the mucus culture plus a gain over the original length.

These tests indicate quite conclusively that the effects exerted by the inhibitory factor are nutritional in nature, and are only temporary.

DISCUSSION

At the beginning of this study, it was thought that the phenomenon of age resistance of a host to its intestinal parasites might be explained on the basis of the findings of Ackert and Edgar (1938) in their histological study of the habitat of A. galli. The close relationship between the increase in the numbers of goblet cells per unit area of intestine as the host ages, and the natural development of age resistance of the host led to the inference that age resistance may be dependent upon the relative amounts of mucus present in the lumen of the intestine. This suggested no qualitative differences in mucus of different ages; only a quantitative difference.

Proceeding on the assumption that there were no qualitative differences in mucus of varying ages, the tests run to determine the effects of mucus from resistant chickens on A. galli cultured in vitro indicated that autoclaved mucus contained a substance that inhibited the growth of the worms. This suggested that the mucus may at least exert certain mechanical effects resembling crenation on the worms. When this test was repeated with autoclaved mucus from young susceptible chickens in order to determine whether this property of inhibition was a characteristic of mucus of all ages, or only of mucus from older chickens, it was found that whereas old mucus had the ability to inhibit the growth of the worms in vitro, young mucus exerted little or no effect. The results of a comparative study on autoclaved mucus of chickens of varying ages indicated that the mucus increased in potency as the chick aged. These findings led to the hypothesis of there being qualitative differences between mucus of chickens of one age from that of fowls of another age.

There are certain points in favor of each of these hypotheses. The first, based on the assumption that the resistance of older chickens is due to the increased amount of mucus in the lumen of the intestine, would apply to those cases in which infection occurred in the susceptible phase of the host's life. However, this cannot be demonstrated in vitro. The second hypothesis, readily demonstrated outside the host, would apply to those instances observed by Herrick (1926) in which infection was attempted in the resistant phase of the host's life. It may be that both methods are factors in the development of resistance

of the host to the parasite, but since the first cannot be readily demonstrated, only the second, or qualitative differences in mucus of different ages will be considered in detail.

The emphasis in this problem has been placed on the tests run on unautoclaved mucus. This has been done primarily because statistical evidence, which is lacking for the earlier tests, can be offered for this phase of the work. The data on the autoclaved material, while highly indicative, were not conclusive, because the numbers of samples were too few. However, when the experiment was repeated with untreated mucus, results of the same nature as those of the early tests were obtained; and in this case they proved to be highly significant by statistical analysis.

Since, however, the significant results were obtained with the tests on untreated mucus, the inference might be made that an antigen-antibody mechanism was the prime factor in the development of the immunity of the host to the parasite. But since the trend of both series of experiments was practically the same, the possibility of an antigen-antibody relationship entering in may be ruled out. This statement is made with one reservation, for in the test on unautoclaved mucus, the inhibition resulting from treatment with 40-60-day mucus was found to be greater than any of the other ages except the 120-140-day group; the group which would be expected to exhibit the greatest inhibition. Part of the mucus used in the 40-60-day group had been taken from chickens which had been parasitized just long enough so that antibodies, if they should be formed, would still be present. This is not meant to imply that antibodies were produced, but the data on

this group did indicate that some other mechanism in addition to that factor which has been shown to develop independent of previous infection, is present in mucus of this age range provided that infection occurred at the proper time. It was because of these results that the data from this age group of mucus was deleted from the final analysis.

The technique and methods involved in this experiment have been described elsewhere in this thesis. Statistical treatment of the data by analysis of variance showed conclusively that there was a difference between the ages of mucus used in the experiment. To determine where this difference might be, a T test was run on the group means using the data from the analysis. This test showed that while the differences between the means of the 60-80, 80-100, and 100-120-day groups do not differ significantly among themselves, the difference between the 120-140-day group and any other group was highly significant.

Tests with autoclaved mucus had indicated that there was a definite increase in the potency of the mucus as an inhibitor as the age of the chick increased. This was not so evident in the experiment under consideration. It is possible that the high degree of interaction of the worms obscured any great significance.

The results of these experiments detract further from the first hypothesis proposed in this problem. Ackert and Edgar (1938) in counting the goblet cells of the intestine by the use of specially prepared slides found that the number of goblet cells per unit area of intestine increased from about 2.9 in chicks two

days of age to about 8.7 in 58-day old birds. After this time there was no great increase in numbers of goblet cells even in chickens up to 320 days of age. Considering only a part of these age counts, a tabular comparison, as given in Table 11, of these counts matched with the group sums of the inhibition, resulting from treatment with mucus from chickens of comparable ages, showed that the results of the experiment now being discussed cannot be correlated with any degree of success with the goblet cell counts.

Table 11. Table showing lack of correlation between goblet cell count per area and inhibition of nematode growth resulting from treatment with mucus from chickens of comparable ages.

Age of Chicken Days	Number of Goblet Cells	Group Sum
58	8.7	- 6.3135
71	9.3	-10.0868
124	10.7	-11.7708
131	9.2	-29.0917

The only possible explanation for these results must be based on the assumption of the presence in the mucus of older chickens of some as yet unknown substance. These results, together with the results obtained by Herrick (1926), quite successfully refute a hypothesis that the resistance of chickens to A. galli is based only on the increased amount of mucus in older chickens.

in their life cycle when their rate of growth was at its peak. Of these three possibilities the last is the most logical. Ackert (1931) in his study of the morphology and life history of _A. galli_ found that the rate of growth of the worm _in vivo_ was greatest as the worm approached maturity. At that time the rate of development is greater than at any time either before or after that period. The first explanation is entirely possible, but should it actually occur, the significance of the test would not be impaired since the same solution was used as a base for both the control and experimental cultures, and the same relationship would therefore exist between the experimental and control worms.

This difference of growth rate was also observed in the 60-day group of worms in the experiment on untreated mucus, except that in this case the reverse occurred, for the loss of length in the 60-day worms resulting from treatment with the mucus was found to be greater than the loss occurring in either the 50 or 70-day worms. It is true that in the cases discussed previously the worms were cultured in comparatively young mucus, while in the second case a greater amount of old mucus was used. However, it is more logical to assume another explanation for this discrepancy. Ackert's work (1931) showed that the growth rate of the parasite increased as maturity was approached. Sixty-day worms are just within the lower age limit of that period. The increased growth rate is a result of the speeding up of physiological reactions brought about by approaching maturity. The assumption can then be made that perhaps the delicate metabolic balance is upset by the presence of the inhibitory factor,

the effects of this disturbance being manifested in a relatively greater loss of length. Possibly this type of reaction might have been observed in the treatments with autoclaved mucus had a sufficiently large number of samples been run.

The results of the tests with treated mucus indicated that the inhibitory ability of the mucus apparently developed very rapidly. This substantiated the results obtained by Ackert, Porter, and Beach (1935) on in vivo studies of the development of resistance. These workers found that even a difference in age of one week between chickens definitely inhibited the growth of the nematodes. This rapid development of resistance was not evident in the comparative study on untreated mucus since the data proved to be nonsignificant when the mucus was considered by 10-day intervals. However, an actual significance may have been obscured by the high degree of interaction between the worms and the mucus.

It will be noted in the general summary of data of the experiment on untreated mucus (Table 4) that many of the tests involving young mucus resulted in a loss of length approximating that occurring with the treatment with old mucus. However, the tests with treated mucus had indicated that young mucus exerted little or no effect on the worms. Of course, it is possible that this discrepancy might have been due to the small number of samples run in the tests on autoclaved material. But to explain this inhibition with young untreated mucus the following hypothesis is offered. The medium used in these tests was essentially a 0.649 percent saline solution with the addition of known amounts of

$MgCl_2$, $CaCl_2$, KCl and dextrose, making the whole medium equivalent in concentration to a 0.832 percent saline solution. Since albumins and globulins are soluble in weak solutions of NaCl, the medium would tend to dissolve out these proteins should they be present in the mucus. Thus, with the addition of mucus to the medium, the albumins and/or globulins would go into solution and would possibly exert some effect on the worm.

But the fact still remains that when all the data are considered a relatively greater loss of length resulted when the worms were treated with old mucus. This may have been due to the presence of some as yet unknown substance in the soluble fraction of the mucus whose concentration and resultant effectiveness increase as the chicken ages, or it may have been due to an increased concentration of soluble proteins in old mucus. At present data are lacking on the ratio of soluble proteins to other mucus constituents. The fact that a constant amount of mucus was used in all tests, and that the effectiveness of the inhibiting agent varied directly with the concentration, substantiate either of these hypotheses as far as the tests with untreated mucus are concerned. But when the tests with autoclaved mucus are considered, the possibility of the soluble proteins acting as an inhibiting agent was ruled out due to the fact that these proteins would be coagulated by autoclaving. Since coagulation by heat is an irreversible process, the albumins and globulins could no longer exert any effect. Therefore, the active inhibiting agent in autoclaved mucus necessarily must be the unknown substance present in the soluble fraction of the mucus. Since the trend

of the experiments on both the treated and the untreated mucus was essentially the same, it is only logical to assume that the development of age resistance of chickens to A. galli is dependent upon the presence of this unknown substance in the mucus.

Thus far in the study it has been shown conclusively that there is some qualitative difference which differentiates between young and old mucus. Tests run to determine in which fraction of the mucus the inhibitory agent was contained showed that it is apparently contained only in the fraction soluble in the medium. This limits it to the fraction containing the soluble proteins.

The procedure used in the above tests has suggested a more efficient method of using the mucus. Heretofore, the use of unautoclaved mucus has entailed a great deal of extra work. It was extremely difficult to prevent lysis of the mucus, and it was impossible to recover the mucus in a sterile condition due to the presence of great numbers of bacteria in the intestine. However, this test has shown that if the mucus is allowed to soak at least 9 days, or possibly a shorter time, all of the inhibitor-containing fraction will go into solution. This washing or leaching can be carried out at low temperatures which will reduce bacterial action to a minimum, and following leaching, the mixture can be filtered to remove all microorganisms. The bacteria-free filtrate may then be used as was the whole mucus-medium mixtures.

In the test to determine whether the effect of the factor is dependent upon concentration, or merely on the presence of

the inhibitory agent, the data indicated that the former is the case. If the latter had been true the same effects would have been observed in all dilutions within certain limits. Instead, when effects are plotted graphically against concentration, the curve follows very closely the theoretical straight line curve which would have been given had effect varied directly with concentration.

The data on the test to determine the effects on the worms of mucus from chickens previously unparasitized but of a resistant age, are offered only for their indicatory value; not as conclusive evidence, since only one test was run. This test indicated that mucus from unparasitized chickens also inhibits the growth of the nematode, *A. galli*. The data indicated also that the inhibitor present in the washings of this mucus could be passed through a Berkefeld filter.

If Herrick's results on *in vivo* studies (1926), Chandler's hypothesis (1939), the findings of Eisenbrandt and Ackert (1940) and the data on the tests involving autoclaved mucus in this problem are all considered, then the results of the test now under discussion possess a definite significance. The workers mentioned above all concur either on the hypothesis that the resistance of fowls to *A. galli* develops independently of previous infection, or that *A. galli* shows little ability to stimulate the host to produce antibodies. With this in mind it is logical to assume the development in the body of the host, independent of previous infection, a secretion in the intestinal mucus, of some as yet unknown agent whose concentration and resultant effectiveness increase with the age of the chick.

Chandler (1935) suggested that the prime factor in the immunity of rats to the trichostrongylid nematode, Nippostrongylus muris, is local, a property of the intestinal mucosa. Chandler further suggested that the immune effect is nutritional in nature and may be due to the development of anti-enzymes on the part of the host which inhibit the highly specialized enzymes by means of which the parasites digest and assimilate the host's proteins. Thus the worms, deprived of their nourishment, have their egg production inhibited if mature, and their development and growth inhibited if immature. Chandler supported this hypothesis with evidence which showed that when stunted adults and non-growing larvae were transferred to a fresh host, the worms were able to resume growth and development as well as egg production (Chandler, 1936). The immune effects of the host on N. muris and A. galli are alike in one respect, i.e., in the retardation of growth. This suggests that the effect of mucus on A. galli is also nutritional in nature.

In order to test this hypothesis, experiments were run which showed that in all but one of the cultures, the worms, after being subjected to the action of immune or resistant mucus for varying periods of time, were able to resume growth when transferred to pure nutrient media. There have been other indications of the recovery reaction of the worms in the regular culture of the nematodes. Very often when the parasites were placed in the pure nutrient medium they were found to give growth rates exceeding that of the animal in vivo. This sudden change of rate of growth might have been due to the reasons given in a previous

section, however it is also logical to assume that it might have been due to the removal of the worm from the presence of the mucus.

These results are similar to Chandler's in two respects: first, that the immune effects of mucus from resistant chickens appear to exert certain nutritional effects; and second, that these effects are only transitory.

In the present work, the opportunity was not offered to determine the results when worms are transferred from young mucus to the nutrient solution, or from old mucus to young mucus. However, on the basis of the results of the test discussed above, and what has already been said about the effects of young mucus, recovery would be expected in each case.

SUMMARY AND CONCLUSIONS

Following two and one half years of intensive study on a possible mechanism responsible for the phenomenon of age resistance of chickens to the intestinal nematode, _Ascaridia galli_, the following conclusions have been reached.

1. Evidence is offered supported by statistical analysis, that duodenal mucus from old, resistant chickens differs both qualitatively and quantitatively from mucus of young chickens susceptible to the parasite.

2. This difference in mucus of different ages is manifested in the ability of the old mucus to inhibit the growth of _A. galli_ when added in constant proportions to _in vitro_ cultures of the worm.

3. Tests with a constant amount of mucous have shown that the efficiency of the mucus as a growth inhibitor varied directly with the age of fowl from which the mucus was taken.

4. The results of a number of separate tests have shown that the inhibitory agent present in duodenal mucus is not of the nature and character of an antibody since mucus which had been autoclaved at 15 lbs. pressure for 20 minutes remained as effective as untreated material. Autoclaving at this pressure, which is equivalent to a temperature of 125° C is known to readily destroy antibodies.

5. While the efficiency of the mucus from older chickens as a growth inhibitor, and the increase in number of goblet cells per unit area of intestine as the chick ages are undoubtedly associated, they cannot be perfectly correlated. The results of experiments have shown that while there is very little difference between numbers of goblet cells in 58, 71, 124, and 131-day old chicks, the inhibitory factor increases markedly in effectiveness during this same interval.

6. Extraction and filtration of the mucus have shown that the inhibitory agent is entirely contained in the fraction of the mucus which is soluble in 0.832 percent saline.

7. A titration of the mucus has indicated that the effectiveness of the inhibitory agent is directly dependent upon concentration.

8. The inhibitory factor appears not to depend on previous or current infection.

9. Evidence is offered which indicated that the effects

exerted on the worm by the inhibitory agent are nutritional in nature.

10. When the results of this problem together with the findings of Herrick, Chandler, Eisenbrandt, and Ackert, and other workers are all considered, two closely related hypotheses are suggested as to the possible mechanism of age resistance of a host to its intestinal parasites.

The first of these is based on the assumption that at about the age of four weeks there begins to develop in the tissues of the host in the region of the habitat of the parasite, some as yet unknown substance which is secreted with the products of the goblet cells. As the normal, well-fed host ages, the concentration and resultant effectiveness of the factor increases until its peak is reached at about the age of 140 days. At this time the resistance is at a maximum.

The second hypothesis may be assumed to be based on certain possible changes in the chemical nature of the secretory products of the goblet cells; this change being brought about by the aging of the host.

In neither case would the development of resistance be dependent upon previous or current infection.

In contact with the parasite, the action of the unknown substance or the age-affected mucus would be manifested in an inhibition of the growth of the worm. This reaction is apparently nutritional in nature and would continue only as long as the worm was in the presence of the inhibiting agent.

11. During this study, certain techniques were developed for problems of this nature. These include a new method for the measurement of the living worms, a nematode _in vitro_ culture medium, and a new method for the use of unautoclaved duodenal mucus.

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